High-dose intravenous immunoglobulin (IVIG) as a modulator of complement activation:

Rationale for use in COVID19.

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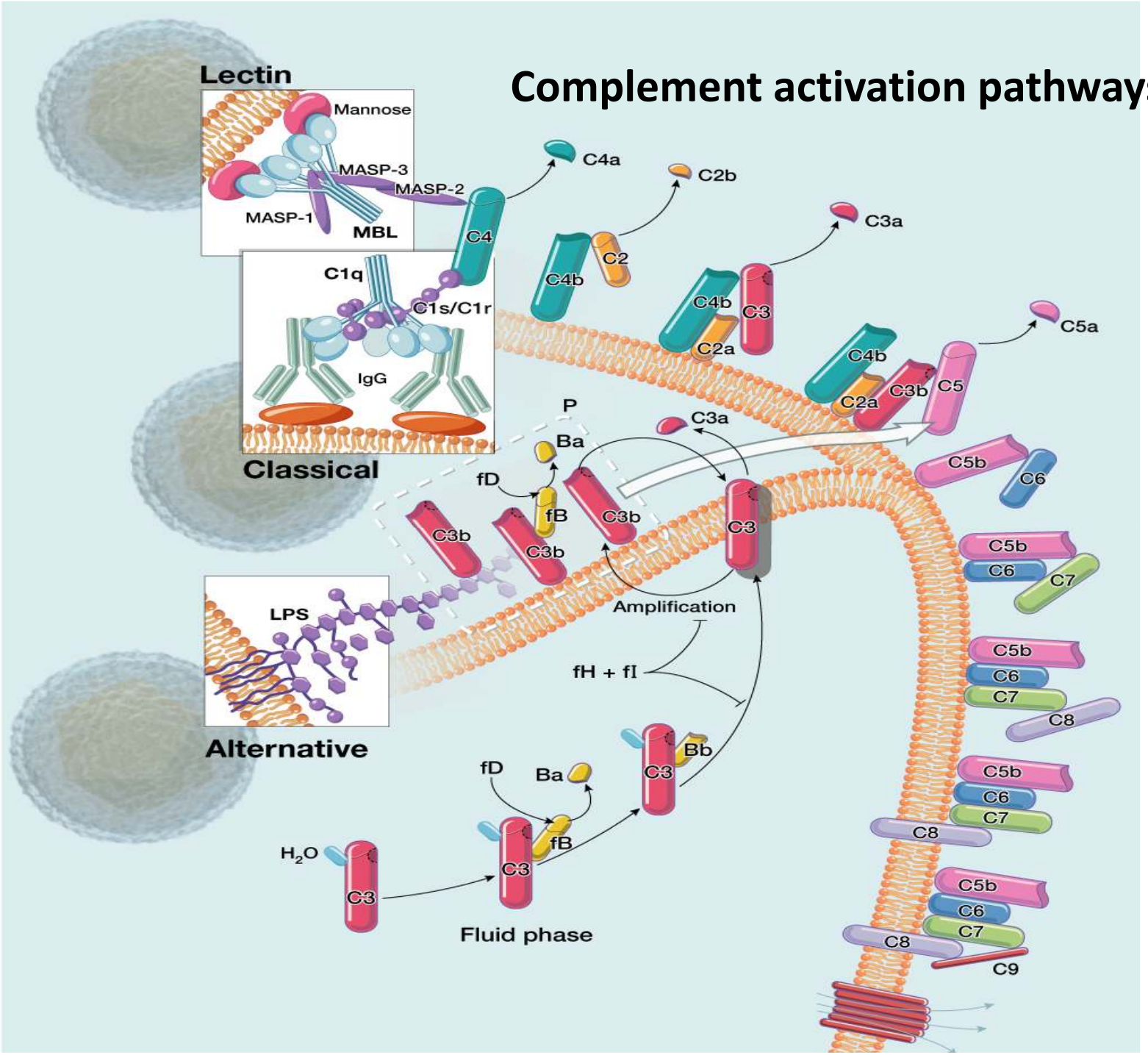
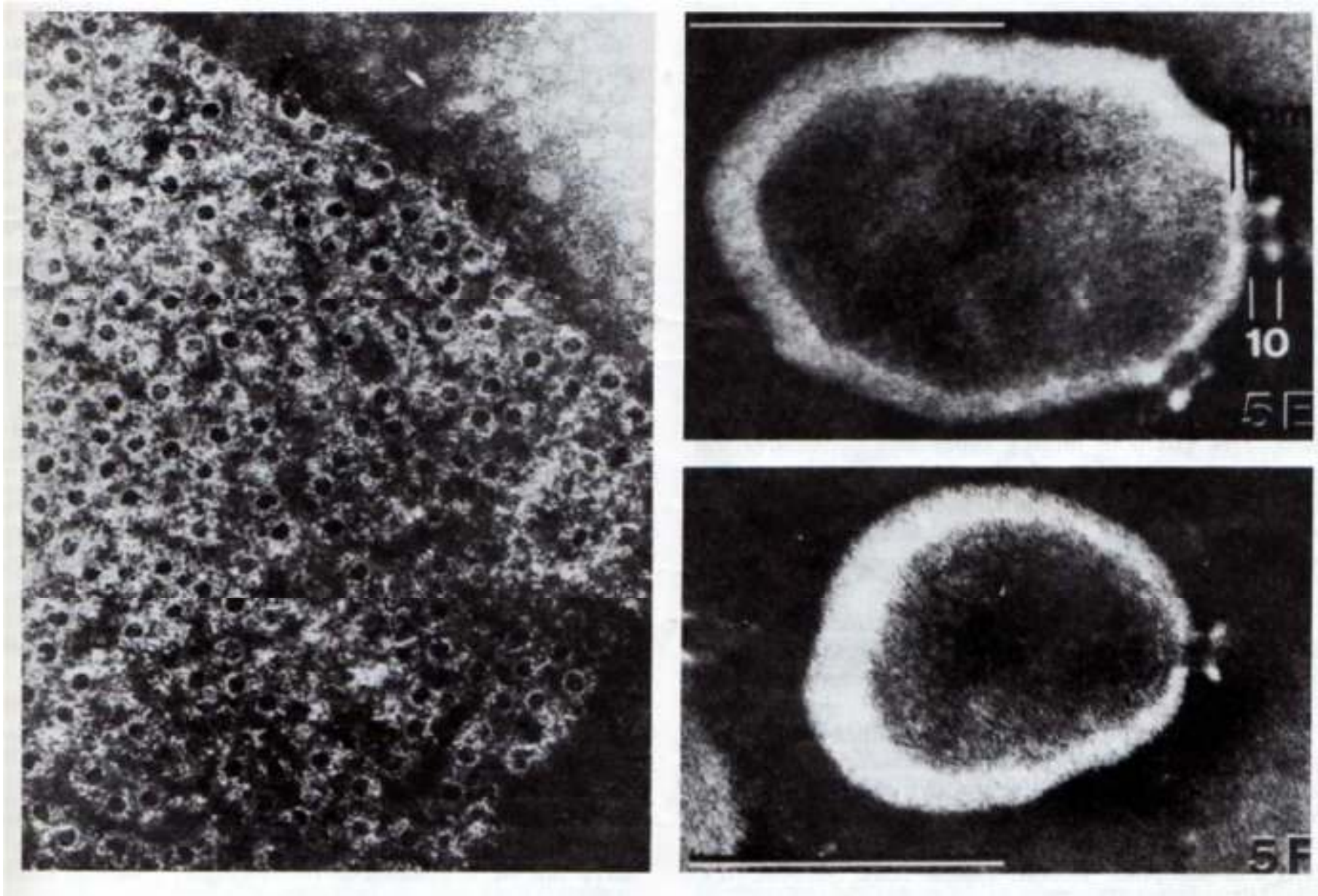
Complement activation pathways

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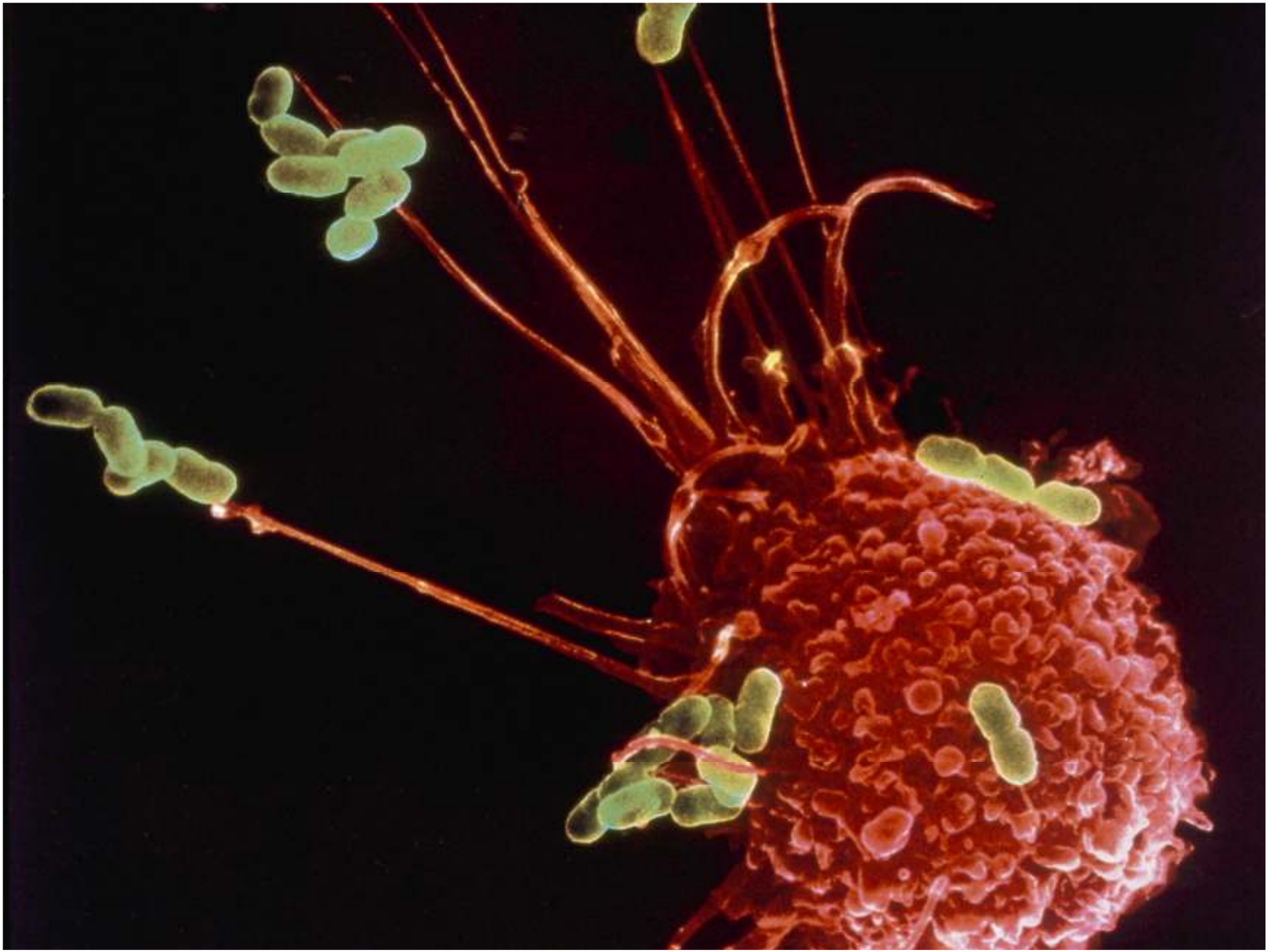
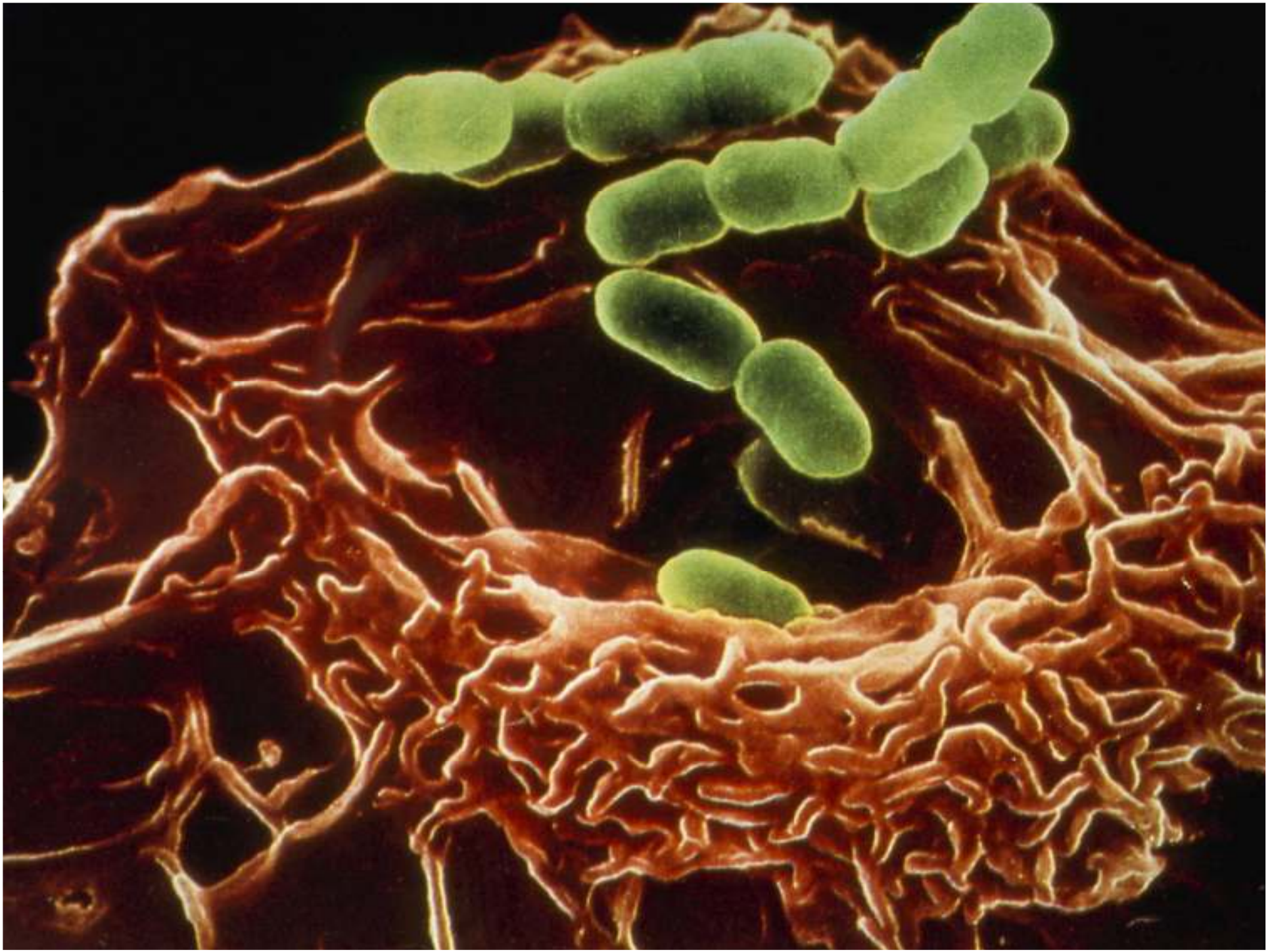
Consequences of Complement activation

- Cell destruction (MAC complex formation)
- Opsonophagocytosis (C3b/C4b)
- Initiation of Inflammation (iC3b, C3a, C5a)
- Amplification of Inflammation (C3a, C5a)
- Activation of the coagulation system





Initiation of Inflammation

- iC3b – up-regulates expression of adhesion molecules on neutrophils(MAC-1) and endothelial cells (ICAM-1)
- C3a- increased vascular permeability, blood vessel smooth muscle contraction, mast cell degranulation/histamine release, chemotaxis
- C5a- same as C3a, 1000-fold more potent, in addition induces endothelial and glial cell activation

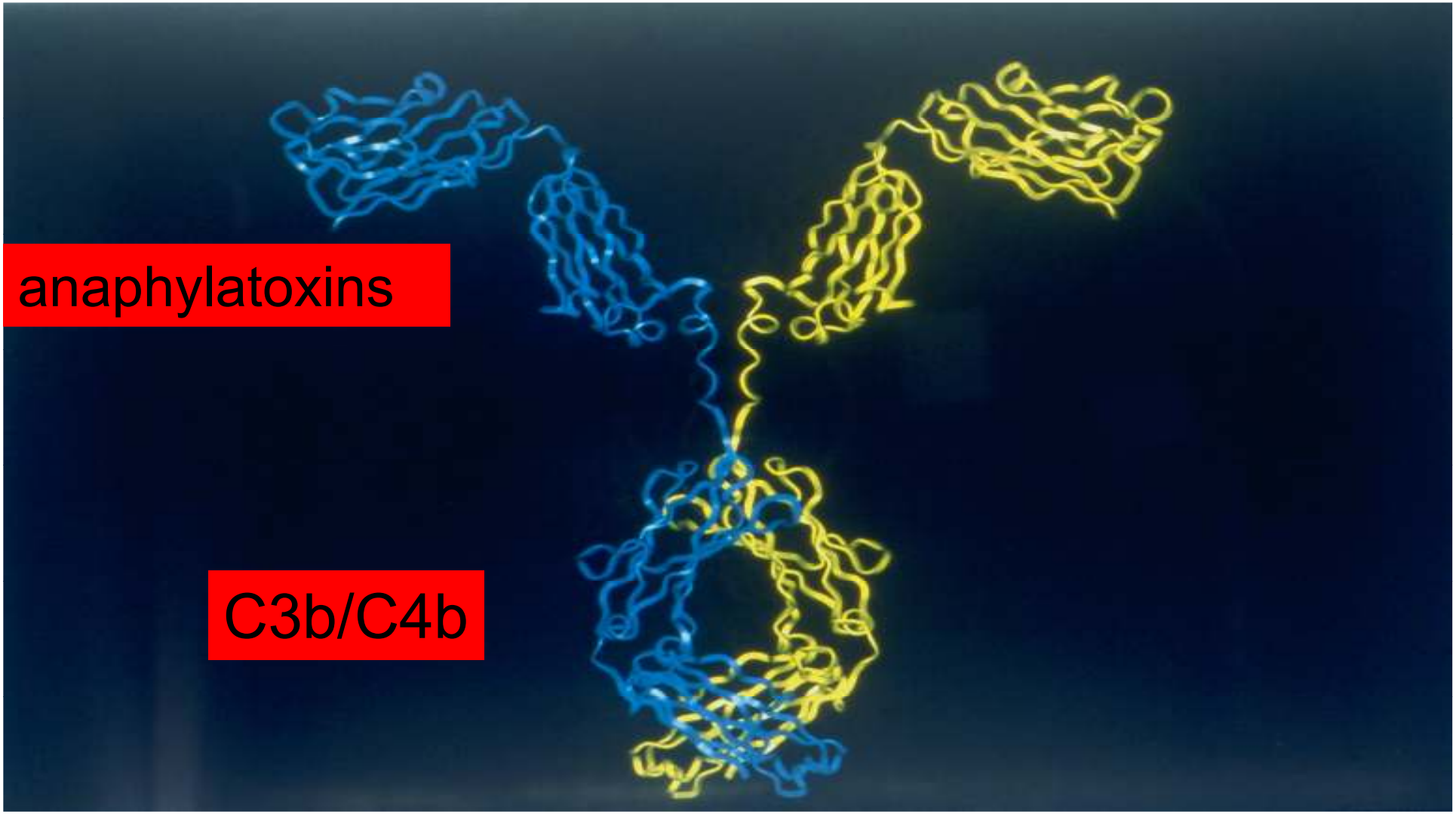
Amplification of Inflammation

- C3a/C5a stimulate production of proinflammatory cytokines (TNF-α, INF-γ, IL-1β, IL-6, IL-8)

  Activation of the coagulation system

  C5a up=regulates plasmin, TF (tissue factor, a cofactor for coagulation factor VIIa) expression on endothelial cells and neutrophils and activates platelets. This results in micro-clots, thrombotic microangiopathy and manifestations of DIC, bleeding and thrombocytopenia

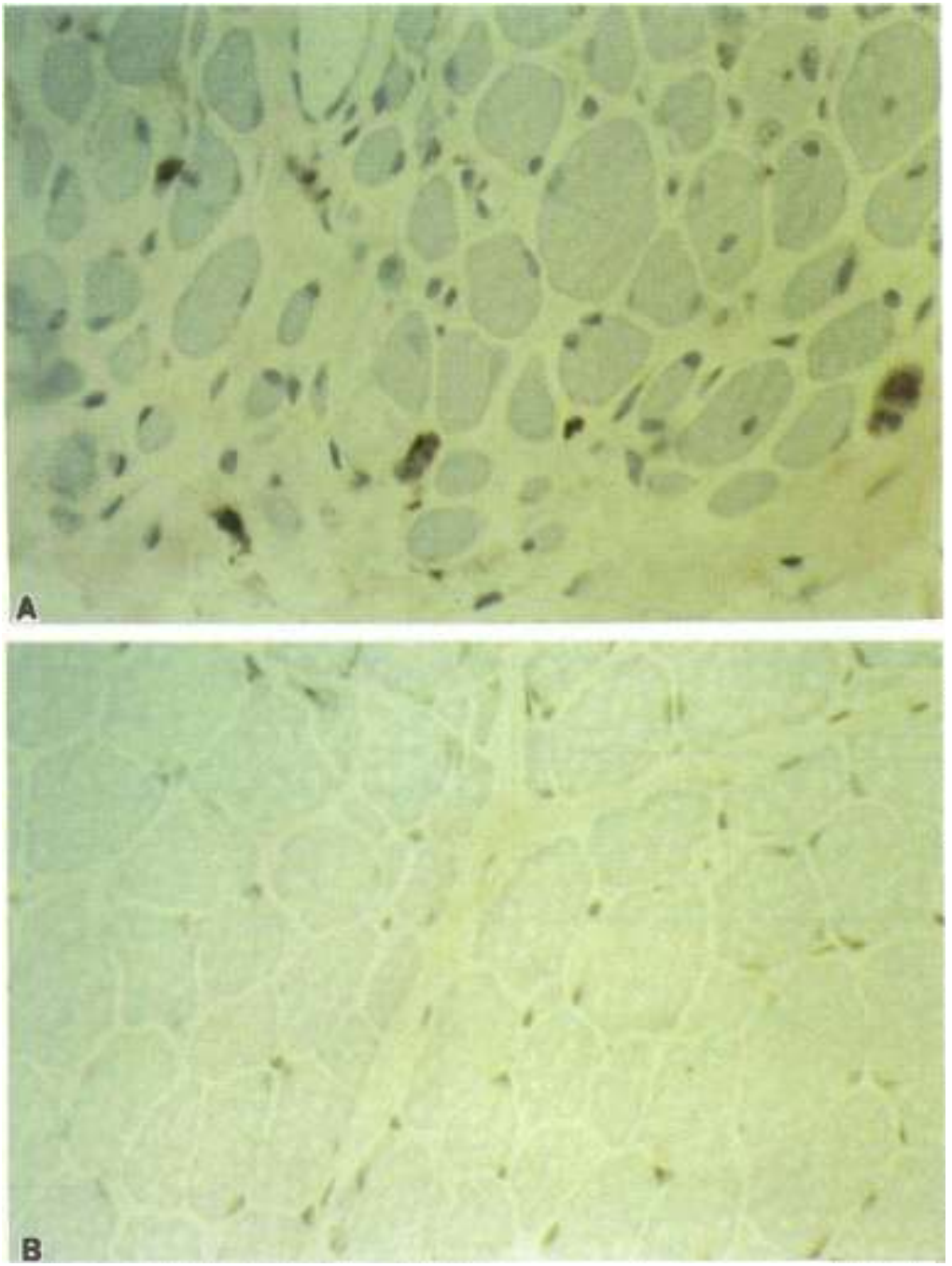
C scavenging is mediated by different regions of the IgG molecule

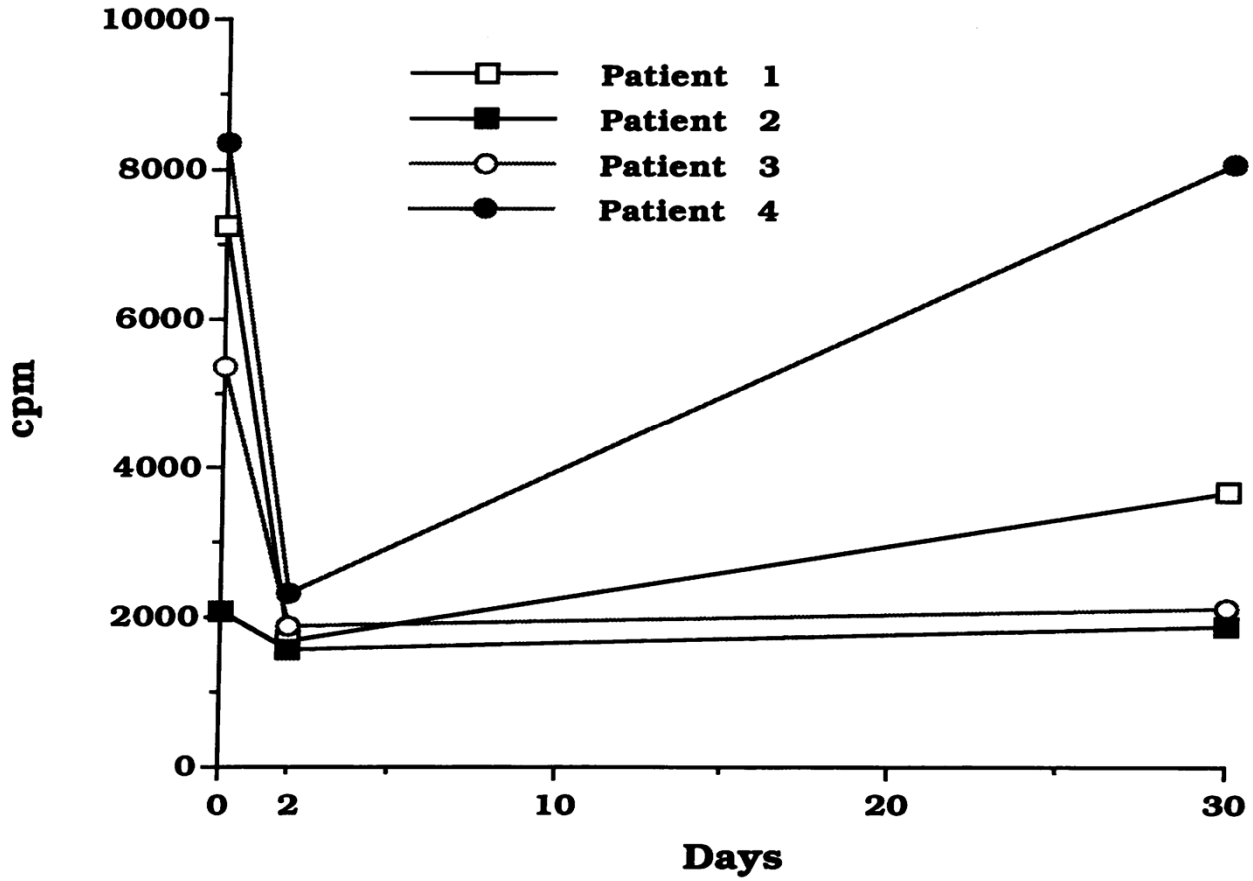


Immunostaining of muscle biopsy specimens from DM patients for C3b NEO antigen.

In the pre-IVIG biopsy, C3b NEO is deposited on the muscle capillaries and some muscle fibers (upper panel);

in the post-IVIG specimen (lower panel), no C3b NEO deposits were detected

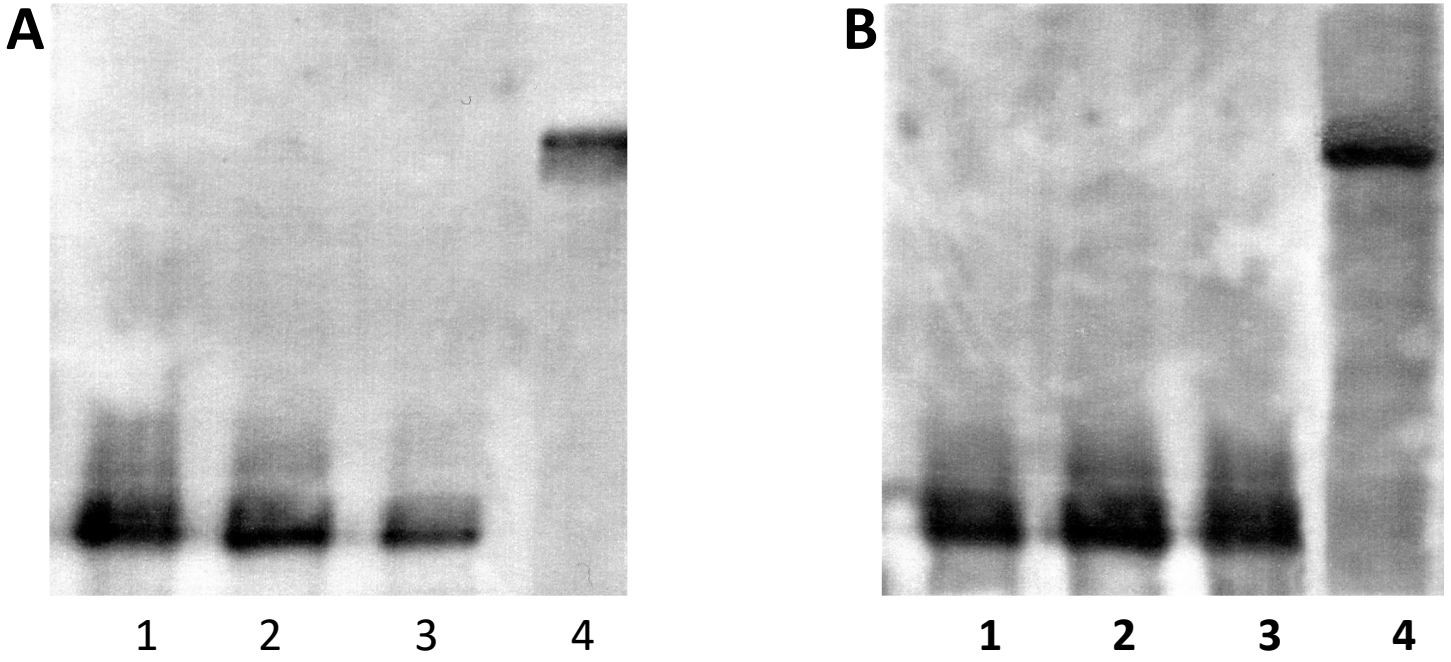




Kinetics of C3b in sera of DM patients after IVIG therapy.

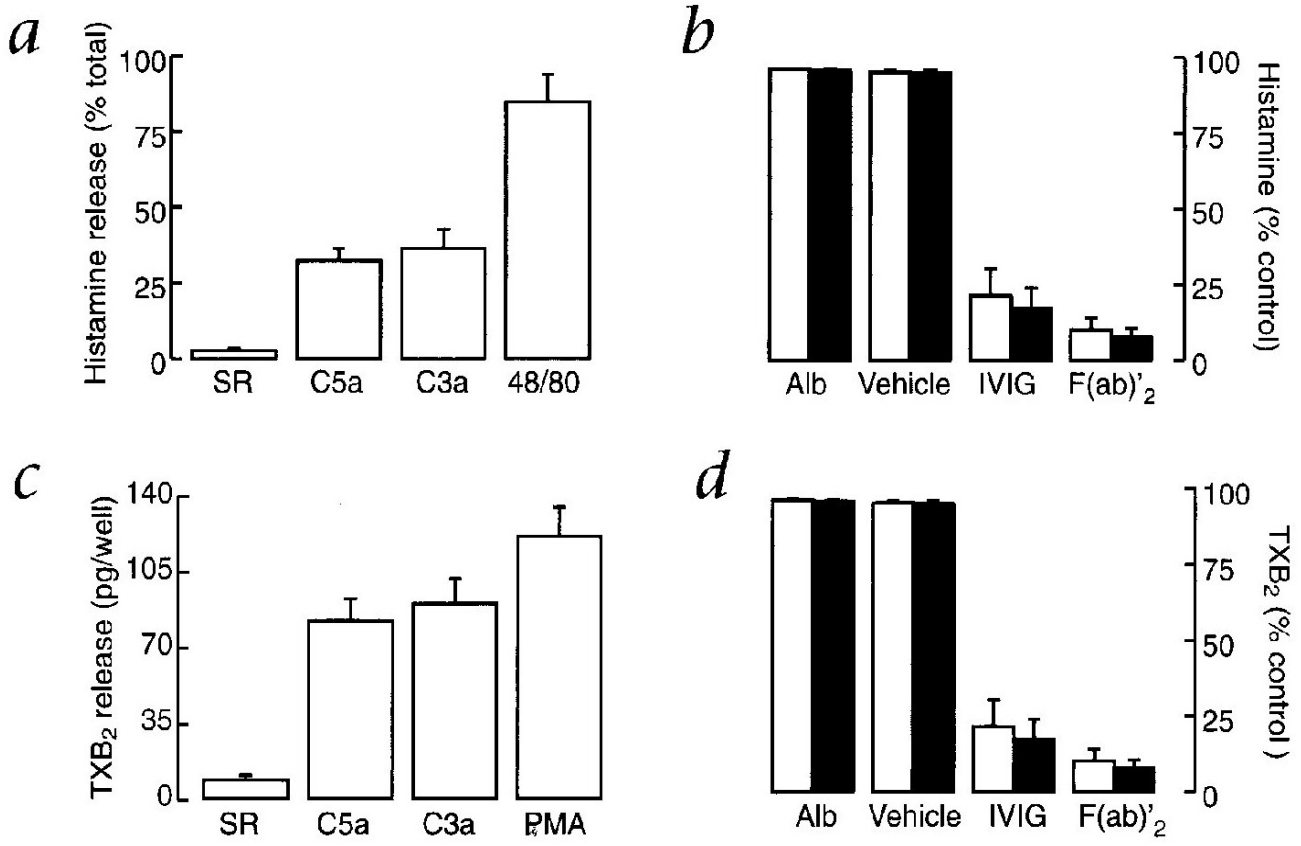
C3b levels were quntitatedin in serum samples of four patients at baseline (day 0), 2 days after completion of IVIG therapy, and 30 days later. At day 2 after IVIG infusion, the C3b value in
all the patients was suppressed to the background level; 30 d later, the C3b values had rebounded as function of the catabolism of infused IgG molecules

Evidence of physical binding of C3a/C5a and $F(ab)_2$

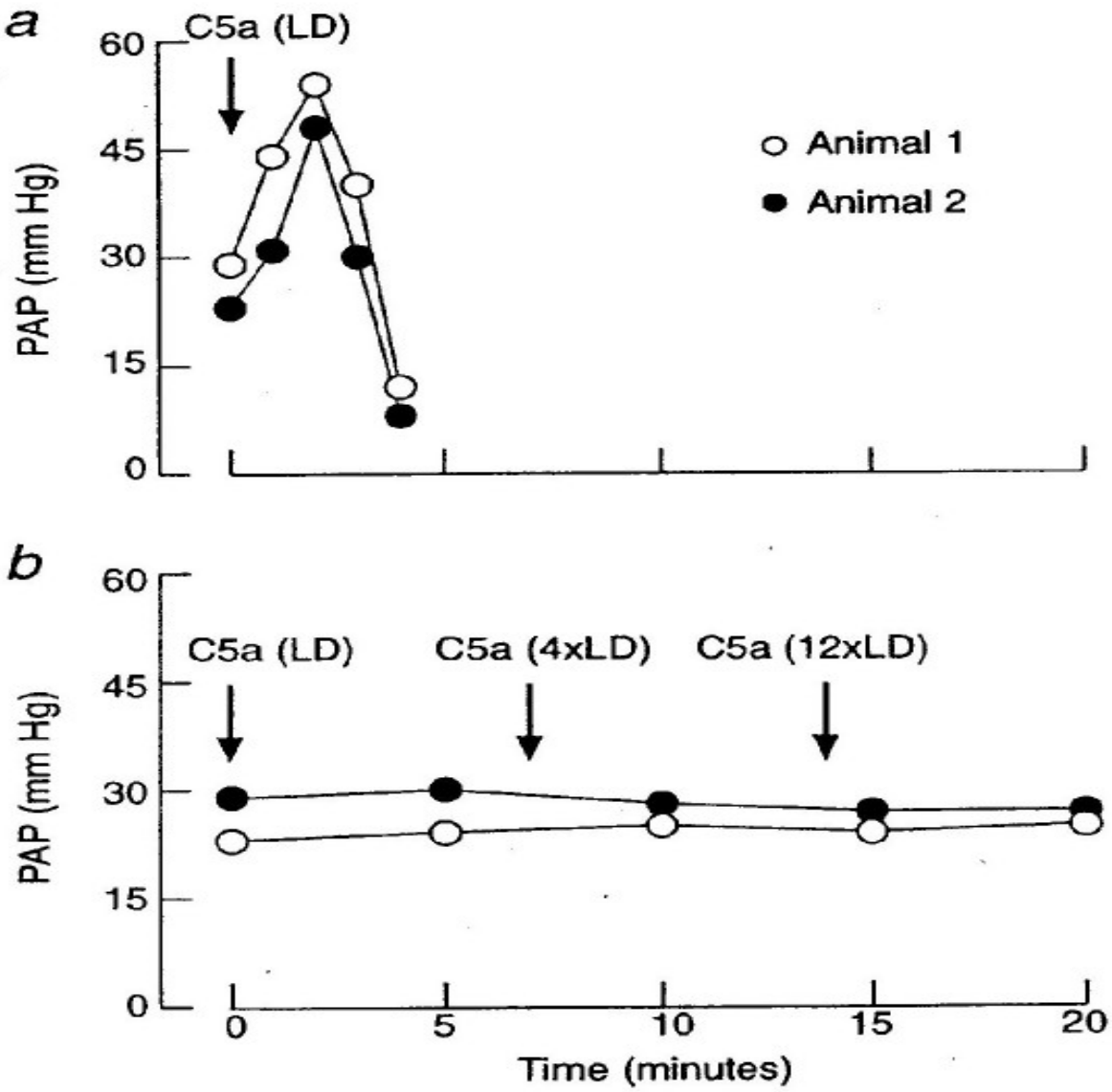


(A)Western blot analysis of C3a–F(ab) complex formation. Lanes were loaded with F(ab) alone (1), C3a pre-incubated with Fc fragment of IgG (2), C3a pre-incubated with albumin (3) and C3a pre-incubated with F(ab)(4). (B) Western blot analysis of C5a–F(ab) complex formation. Lanes were loaded identical to Panel A, only C3a was replaced with C5a. **Molecular weight of the bands in lanes 4 indicate formation of respective C3a/C5a and Fab complexes.**

Inhibition of Histamine/Throboxane Release from HMC-1 cell



In-vivo neutralization of C5a by IVIG



The concept of C scavenging

- Supraphysiologic concentrations of IgG molecules prevent complement fragments from binding to their targets.
- In doing so, IgG engage different structrual regions –constant domain of Fab to bind and neutralize anaphylatoxins and Fc fragment to bind C3b/C4b
- Consequently, assembly of the membrane attack complex (C5b-9) is interrupted as well as opsonophagocytosis and complement-mediated hyperinflammation and activation of coagulation

Complement activation on full display in COVID

- In COVID,there is evidence of complement activation and high levels of C5a, sC5b-9 and other active fragments, subsequent inflammation and augmented inflammation (cytokine storm) as well as activated coagulation system with disseminated thrombotic microangiopathy

Re-purposing IVIG for Covid

- IVIG should be added to the arsenal of COVID therapies
- Most hospitals' pharmacies have those preparations, so it is available for immediate off-label use upon patient consent. The best IVIG preparation to administer? The one that is available. If in a position to choose, 10% and IgM-enriched would be slightly favored.
- We are currently using Pentaglobin, an IgM/IgA enriched IVIG in Serbia with encouraging results
- We are also testing the complement scavenging mechanism of beneficial effect.

In summary

- Complement is activated in COVID and mediates lung and multi-organ injuries, systemic hyperinflammation (cytokine storm) and disseminated thrombotic events.
- Immunolgobulin molecules present in IVIG preparations have the capacity to bind and neutralize activated complement fragments responsible for the above-mentioned pathologies;
- IVIG, therefore, is a logical therapeutic choice in COVID

For any further questions, discussions or advice, here is my contact information:

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Zoom

I can provide help with the treatment protocol, especially timing of IVIG infusion, dosing and rate of infusion, all of which are critical for achieving the beneficial effect